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(54) Title: PRODUCTION PROCESS FOR INTRAVENOUS IMMUNE SERUM GLOBULIN AND RESULTANT PRODUCT

(57) Abstract

A process for producing an intravenously-administrable gamma globulin solution substantially free of contaminating viruses by heat treating for viral inactivation and fractionating an impure gamma globulin solution and then treating the purified gamma globulin with a solvent-detergent for further viral inactivation.

PRODUCTION PROCESS FOR INTRAVENOUS IMMUNE SERUM GLOBULIN AND RESULTANT PRODUCT

BACKGROUND OF THE INVENTION

The present invention relates to an integral, multi-step commercial process for the production of intravenously administrable immune serum globulin containing IgG (γ-globulin) as the main ingredient.

Various processes are known for obtaining intravenously administrable γ-globulin solutions from starting materials resulting from Cohn fractionation of human plasma. Certain of the Cohn fractions contain higher titres of γ-globulin than others. Usual starting materials for a γ-globulin solution are Cohn Fraction II or Cohn Fraction II + III.

Although prior art processors employ various separation and sterilization techniques, process modifications are constantly sought for improving final product purity and safety, and overall yield.

Many commercial processes employ either a solvent/detergent step for viral inactivation, or a heat treatment step for viral inactivation. To date, the art has not provided a multi-step process

beginning with Cohn Fraction II paste or II + III paste including two different viral inactivation procedures as part of an efficient, high yield γ-globulin manufacturing process.

U.S. Patent 5,151,499 by Kameyama et al. is directed to a process for producing viral inactivated protein compositions in which a protein composition is subjected to a viral inactivation for envelope viruses in a solvent/detergent treatment of the protein composition and a viral inactivation for non-envelope viruses in a heat treatment of the protein composition. The '499 patent teaches that preferably the solvent/detergent step occurs first and in the presence of a protease inhibitor, followed by a heat treatment. Where the heat treatment is carried out in the liquid state, the protein is first recovered from the solvent/detergent by adsorption onto an ionic exchange column, prior to any heat treatment. The liquid heat treatment can be carried out in the presence of a sugar, sugar alcohol or amino acid stabilizer. Although the '644 patent lists many starting protein compositions including immunoglobulin, its production examples employ Factor IX, thrombin, fibrinogen and fibronectin.

Removal of denatured protein produced in a heat treatment step through fractionation is not considered.

Certain prior art processes for production of intravenously injectable γ-globulin solutions describe the incorporation of a liquid heat treatment carried out in the presence of sorbitol heat stabilizer in a multi-step purification procedure beginning with Cohn Fraction II + III paste. In U.S. Patent 4,845,199 by Hirao et al., Cohn Fraction II + III is subjected to polyethylene glycol (hereinafter "PEG") fractionation (8% w/v PEG followed by 12% w/v PEG), then ion exchange chromatography (DEAE-Sephadex) and removal of human blood group antibody prior to a liquid heat treatment in the presence of sorbitol as a protein stabilizer. On the other hand, Example 1 of U.S. Patent 4,876,088 by Hirao et al. describes the preparation of intravenously injectable γ-globulin solution from Cohn Fraction II + III paste in which the paste is suspended in water, its pH adjusted to 5.5 and centrifuged, with the supernatant then being heat treated for viral inactivation in the presence of 33% w/v of sorbitol, followed by PEG fractionation (6%/12%) which would remove heat denatured protein and

then by other purification steps including DEAE-Sephadex ion exchange chromatography.

SUMMARY OF THE INVENTION

In one embodiment the present invention provides an integral, commercially useable process for producing a highly purified γ-globulin solution from the Cohn fractionation process.

Another embodiment of the present invention provides very pure intravenously administrable γ-globulin solution free of both envelope and non-envelope viruses, including all heat sensitive viruses.

A further embodiment of the present invention provides a commercial γ-globulin process enabling removal of any denatured protein produced during heat sterilization prior to a second stage viral inactivation.

In one embodiment the present invention provides a process for preparing an intravenously administrable gamma globulin solution which comprises:

(a) heat treating an impure gamma globulin solution under time and temperature conditions sufficient for inactivating heat sensitive viruses;

(b) subjecting the heat treated gamma globulin

solution to polyethylene glycol fractionation for obtaining a purified gamma globulin solution;

(c) treating the purified gamma globulin solution with an organic solvent for inactivating envelope viruses; and

(d) treating the organic solvent treated gamma globulin with a cationic exchange resin.

In a preferred embodiment of the present invention, an alcoholic Cohn fraction, which may be partially purified, but is rich in γ-

globulin, is heat treated in aqueous medium in the presence of a heat stabilizer for viral inactivation and is thereafter first subjected to PEG fractionation, and then to a second viral inactivation in the presence of a solvent, preferably a solvent-detergent mixture, for disruption of envelope viruses, followed by separation from the solvent or solvent-detergent mixture.

In a preferred embodiment of the present invention, sorbitol is the heat stabilizer and trialkyl phosphate is the solvent.

In another preferred embodiment of the present invention, denatured products of the heat treatment viral inactivation are removed by the PEG fractionation prior to the second viral inactivation for providing an exceedingly pure heat treated γ-globulin.

In another preferred embodiment of the present invention, any particulates present are removed prior to the solvent-detergent treatment.

In still another embodiment of the invention, there is provided a heat-sterilized and solvent-

detergent sterilized γ-globulin suitable for intravenous administration.

DETAILED DESCRIPTION OF THE INVENTION

A fraction containing immunoglobulin is used as the starting material. This fraction is not particularly limited in so far as it originates from human serum and contains an immunoglobulin fraction. Specific examples of such an immunoglobulin-containing fraction include Fraction II + III and Fraction II obtainable by ethanol fractionation of Cohn, and paste of immunoglobulin-containing fractions equivalent thereto. Other starting materials are Fractions I + II + III, and Fraction II + IIIw. The starting material may contain impurities, such as human blood-group antibodies, plasminogen, plasmin, kallikrein, prekallikrein activator, IgM, IgA, IgG polymers (hereinafter "aggregates"), etc.

The preferred starting materials are Cohn Fraction II or Cohn Fraction II + III. When Cohn Fraction II + III paste is used, it is recommended that it first be subjected to a preliminary washing procedure to form Fraction II + IIIw, which is thereafter used in the process of this invention.

"Fraction II + IIIw" is a disodium phosphate solution-washed Cohn Fraction II + III precipitate.

Fraction II + IIIw can be obtained by suspending Fraction II + III precipitate in cold water for injection in a ratio of about 1 kilogram of II + III paste per about 20 volumes of water. A sodium phosphate solution is added to the final concentration of approximately 0.003M sodium phosphate for solubilizing lipids, lipoproteins and albumin. Cold ethanol is added to bring the final alcohol concentration to about 20%. During the alcohol addition, temperature is gradually lowered to -5±1°C and pH is maintained or adjusted to 7.2±0.1, for example by using acetate buffer or dilute sodium hydroxide. The Fraction II + IIIw precipitate which forms is recovered by centrifugation and/or filtration while maintaining the temperature at -5±1°C.

Prior to the first viral inactivation step of the present invention, various preliminary purification and/or aggregate-reducing steps can be carried out. For example, when Fraction II + IIIw paste is used, typically containing about 20% alcohol and more than 70% IgG, it can be suspended in 3 to 10 volumes, preferably 3 to 5 volumes, of cold water at a

temperature of about 0 to 5°C and with pH being adjusted to be between 4.5 to 6.0, preferably 5.0 to 5.5 using pH 4.0 acetate buffer or hydrochloric acid. The mixture is agitated for about 2 to 15 hours to allow all of the γ-globulin to go into solution. Thereafter, undissolved protein such as albumin and α-globulins can be removed by centrifugation and/or filtration.

Where a different starting Cohn fraction is employed, the initial step or steps of the process can be appropriately selected where desired for carrying out a preliminary purification for obtaining a fraction of high IgG content to be further processed. For example, where Cohn Fraction II (contains over 95% IgG) has been separated from Cohn Fraction III, with Fraction II to be further processed, the initial processing can be at an acid pH of 3.2 to 5.0, preferably 3.8 to 4.2, as described by Uemura et al. U.S. Patent 4,371,520, in order to break down immune globulin aggregates present into immune globulin monomers and dimers, since aggregates are known to possess anti-complementary activity (ACA). As another alternative, with Cohn Fraction II + III starting material, the Uemura, et al. patent low pH treatment can be carried out as an additional step following an

initial purification step as above described and prior to the viral inactivating heat treatment step.

For the heat sterilization step, the immune globulin protein is dissolved in water or, if in the form of an aqueous mixture such as the filtrate collected from the above-described partial purification of Fraction II + III, it can be used as is, and a sugar, sugar alcohol and/or amino acid heat stabilizer is added thereto. The heat stabilizer is preferably sucrose, maltose, sorbitol or mannitol, most preferably sorbitol. The sugar or sugar alcohol is added to the immune globulin solution as a powder or first mixed with a small volume of water and then added, to provide a final concentration of about 10 to 50 w/v%, up to saturation. At this point, the aqueous solution of immune globulin contains sufficient water so that this solution contains about 1 to 6% total protein, a typical Fraction II + III starting material containing about 300 grams protein per kilogram paste.

Following addition of the heat stabilizer, the mixture is heated at about 50-70°C for about 10-100 hours, preferably at about 60°C, for about 10 to 20 hours, for viral inactivation of heat sensitive viruses. The heat treatment step not only inactivates

viruses, but also through the protein denaturization effect thereof, can preferentially reduce the amount of certain undesirable proteins normally associated with Cohn Fractions II + III, such as prekallikrein, plasmin, plasminogen and IgA.

After the heat treatment, cold water to the extent necessary is added so that protein concentration is maintained at about 0.3 to 2.0 %. The solution is cooled to 0-2°C.

Next, PEG fractionation is carried out on the heat treated solution. PEG fractionation is a well known procedure in the art of purification of immune globulin in order to separate the desired IgG monomer and dimer from IgG aggregate and from other impurities naturally occurring in the starting plasma protein fraction. However, in the instant process, the PEG fractionation also accomplishes a separation between the desired IgG monomer and dimer, and unwanted denatured protein products produced by the heat treatment. These denatured protein products are denatured prekallikrein, plasminogen, plasmin, IgA, IgM and aggregates.

Any of the PEG fractionation procedures documented in the prior art can be used. In general, two stages of PEG fractionation are carried out. In the first stage of PEG fractionation, PEG concentration and pH are selected so that the desired IgG monomer and dimer remain in solution while undesired proteins such as aggregate are precipitated out of solution. Following centrifugation and/or filtration, PEG concentration is increased with adjusting the pH to cause the desired IgG monomer and dimer to precipitate.

For example, a first stage of PEG fractionation can be carried out at a pH of about 5.0 to 7.5, preferably within about 6.5 to 7.5 pH when Fraction II + IIIw paste is used as starting material, and preferably within about 5.5 to 6.0 pH when Fraction II + III paste is used as starting material, with a PEG concentration ranging from about 4 to 8%, preferably either 4 to 6% when Fraction II + IIIw paste is used as starting material, or 6 to 8% when Fraction II + III paste is used as starting material. While maintaining cold temperatures of about 0-2°C, the first stage of PEG fractionation can be carried out for about 1 to 8 hours, after which the precipitate is removed as above-described. The

filtrate will then have its pH adjusted to about 8.0 to 9.0, preferably about 8.5 to 8.9, and additional PEG added for final concentration of about 10 to 15%, preferably about 12%. The precipitate formed, which is purified immunoglobulin, is removed by filtration and/or centrifugation.

Further details of PEG fractionation procedures usable in the practice of the present invention can be found in the above-described U.S. Patent 4,876,088 by Hirao et al. and U.S. Patent 4,845,199 by Hirao et al.

The final essential step of the present invention is to carry out a second viral inactivation procedure utilizing a solvent or solvent-detergent mixture. As described below, further purification procedures, specifically those involving the use of ionic exchange resins, can be carried out prior to and/or following the solvent-detergent treatment. A particularly advantageous procedure is to carry out an anionic exchange treatment prior to the solvent detergent viral inactivation and then a cationic exchange treatment after the solvent detergent viral inactivation. By this procedure, certain undesirable protein materials (such as prekallikrein activator, IgA, IgM and albumin) found within human plasma can be

removed from the IgG by use of the anionic exchanger and then further such materials (prekallikrein activator, IgA, IgM, albumin and PEG) along with the residual reagents used in the solvent-detergent treatment can be removed through the cationic exchange procedure.

If not otherwise accomplished during the overall process the solution to be subjected to the solvent-detergent should be treated for removal of all particulate matter, which can include denatured protein. Therefore, it is preferred to filter the solution with a 1 micron or finer filter prior to solvent-detergent addition. This will also reduce the likelihood of virus being present within a large particle and thereby possibly avoiding exposure to the solvent-detergent.

Today, the preferred solvent for inactivation of envelope viruses is trialkyl phosphate. The trialkyl phosphate used in the present invention is not subject to particular limitation, but it is preferable to use tri(n-butyl)phosphate (hereinafter "TNBP"). Other usable trialkyl phosphates are the tri(ter-butyl)phosphate, the tri(n-hexyl)phosphate, the tri(2-ethylhexyl)phosphate, and so on. It is possible to

use a mixture of 2 or more different trialkyl phosphates.

The trialkyl phosphate is used in an amount of between 0.01 to 10 (w/v)%, preferably about 0.1 to 3 (w/v)%.

The trialkyl phosphate may be used in the presence or absence of a detergent or surfactant. It is preferable to use trialkyl phosphate in combination with the detergent. The detergent functions to enhance the contact of the viruses in the immune globulin composition with the trialkyl phosphate.

Examples of the detergent include polyoxyethylene derivatives of a fatty acid, partial esters of anhydrous sorbitol such as Polysorbate 80 (Tradename: Tween 80, etc.) and Polysorbate 20 (Tradename: Tween 20, etc.); and nonionic oil bath rinsing agent such as oxyethylated alkylphenol (Tradename: Triton X100, etc.) Examples include other surfactants and detergents such as Zwitter ionic detergents and so on.

When using the detergent, it is not added in a critical amount; for example, it may be used at ratios

between about 0.001% and about 10%, preferably between about 0.01% and 3%.

In the present invention, the trialkyl phosphate treatment of the immune globulin containing composition is carried out at about 20 to 35°C, preferably 25 to 30°C, for more than 1 hour, preferably about 5 to 8 hours, more preferably about 6 to 7 hours.

During the trialkyl phosphate treatment, immune globulin is present at about a 3 to 8% protein solution in aqueous medium.

If not carried out prior to the solvent-detergent treatment, an anionic exchange treatment can be carried out on the solvent detergent treated immune globulin. Preferably, at least a cationic exchange treatment is carried out on the solvent-detergent treated product. The ionic exchange treatments are carried out with immune globulin dissolved in an aqueous solvent, generally having a pH of about 5-8, with where desired low ionic strength for maximum adsorption of IgG. The protein concentration generally is within the range of about 1-15 w/v%, more preferably from about 3 to 10 w/v%. The ionic

exchanger is equilibrated with the same aqueous solvent as used, and either a batch or continuous system can be carried out. For instance, anionic exchange batch-wise treatment can be carried out by mixing the immune globulin solution with the anionic exchanger in an amount from about 10 to 100 ml per ml of the pretreated anionic exchanger (for example, 1 gram of DEAE Sephadex A-50 resin swells to about 20 grams wet weight in 0.4% sodium chloride solution), stirring the mixture at about 0-5°C for about 0.5 to 5 hours, and then filtering or centrifuging at 6,000 to 8,000 rpm for 10 to 30 minutes to recover the supernatant liquor. Continuous treatment can be affected by passing immune globulin solution through a column of the anionic exchanger at a rate from about 10 to 100 ml per ml of the ionic exchanger and recovering the non-adsorbed fraction.

The anionic exchanger to be used, for example, comprises anion exchanging groups bonded to an insoluble carrier. The anion exchanging groups include diethylaminoethyl (DEAE), a quaternary aminoethyl (QAE) group, etc., and the insoluble carrier includes agarose, cellulose, dextran, polyacrylamide, etc.

Usable cationic exchangers are carboxy methyl Sephadex (CM-Sephadex) CM-cellulose, SP-Sephadex, CM-Sepharose and S-Sepharose. 1 ml of pretreated cationic exchanger (for example, 1 gram of CM-Sephadex C-50 resin swells to about 30-35 grams wet weight in 0.4% sodium chloride solution) is mixed with 0.5 ml to 5 ml of immune globulin solution and stirred at 0-5°C for 1-6 hours. The suspension is centrifuged or filtered to recover the IgG adsorbed resin. Also, a continuous process can be employed.

When the above-described conditions are used with the cationic exchanger, the IgG will be adsorbed, and thereafter following washing of the protein-adsorbed cationic exchange resin, IgG can be eluted, for example by about a 1.4 N sodium chloride solution.

Following the steps of the above process, the IgG is clarified, diafiltered and concentrated to the extent needed. If desired, a stabilizer such as D-sorbitol can be added and final adjustments made to yield a solution of a composition containing about 100 mg/ml IgG, and 50 mg/ml D-sorbitol, with pH being at 5.4. This solution is then sterile filtered through sterilized bacterial retentive filters and filled into vials.

The following examples are set forth to illustrate the invention but are non-limiting.

Where desired, other immune globulin purification procedures can be appropriately combined with the processes described herein. For example, a bentonite clarification step, useful for reducing the levels of kallikrein and pre-kallikrein activator can be employed. An illustration of this is set forth in Example 1, hereinbelow.

Example 1: Heat Treated and Solvent-detergent Treated γ-Globulin

Six hundred and eighty five grams of Fr II + IIIw paste was suspended in about 11.9 kg of cold water. Sodium acetate trihydrate solution was added to the suspension to a final concentration of approximately 0.04M to selectively solubilize IgG. After mixing for about 15 minutes, pH of the suspension was adjusted to 4.8 with pH 4.0 acetate buffer. Cold alcohol (95%) was added to the suspension to a final concentration of 17%. During the alcohol addition the temperature of the suspension was lowered gradually to about -6°C. Three hundred and three grams of acid washed Celite 535 available from Celite Corporation was added as a

filter aid to the suspension to a final concentration of about 2.0%. After mixing for one hour, the Celite and the Fraction III paste containing unwanted protein such as plasmin, plasminogen, IgA and IgM were then removed by filtration utilizing a filter press. The filtrate was further clarified by 0.45 μm and 0.2 μm filters.

The pH of the Fraction II + III w clarified solution was adjusted to 4.0 with 1.0 N hydrochloric acid and then concentrated by ultrafiltration to about 3.4 liters (1/5th the original volume). Cold water equal to the amount removed by the 1st ultrafiltration was added to the concentrated solution and it was again concentrated by ultrafiltration to about 1/5th the original volume. At this step, protein concentration of the solution was about 2%. The solution was further concentrated to about 4% and diafiltered against cold water until the conductivity of the solution was below 300 μS/cm to help avoid protein aggregation and denaturation during heat treatment. The solution was further concentrated to about 8.8% protein. D-sorbitol was added to the solution to a final concentration of about 33%. After mixing for 30 minutes, pH of the sorbitol containing solution was adjusted to 5.5 with 0.5 N sodium

hydroxide. The solution was then heated for 10 hours at 60°C. After the heat treatment, cold water equal to 3 times the volume of the heat treated solution was added and the diluted solution was cooled to 0-2°C.

The pH of the solution was adjusted to 6.9 with 0.25 N sodium hydroxide and 50% polyethylene glycol (PEG) 3350 was added to the solution to give a final PEG concentration of 4%. Sodium chloride concentration of the solution was adjusted to about 8 mM to aid in precipitation of impurities and aggregates at pH 6.9. The precipitate so formed was removed by filtration. The pH of the filtrate was adjusted to 4.8 with 1.0 N hydrochloric acid and bentonite was added to a final concentration of about 0.25%. The pH of the bentonite suspension was readjusted to about 5.2 and then the suspension was filtered to remove bentonite. The filtrate pH was adjusted to 8.5 with 0.25 N sodium hydroxide and 50% PEG 3350 solution was added to a final PEG concentration of 12%. The precipitate so formed (purified immune globulin) was removed by centrifugation.

About 175 grams of purified immune globulin paste obtained as above was suspended in about 790 g of 0.3%

sodium chloride solution. pH of the suspension was adjusted to 5.5 and then the suspension was mixed for 2½ hours. Sixty two grams of previously equilibrated DEAE-Sephadex A-50 resin (with 0.3% sodium chloride at pH 5.5), was added to the solution and the suspension was mixed for 2 hours. The suspension was then filtered to remove the resin. After adjusting the concentration of sodium chloride to 0.4%, tri-n-butyl phosphate (TNBP) and Polysorbate 80 mixture was added to the filtrate to yield a solution with final concentration of 0.3% TNBP and 1.0% Polysorbate 80. After overnight incubation, pH of the solution was adjusted to 5.8 and about 860 grams of previously equilibrated CM-Sephadex C-50 (with 0.4% sodium chloride at pH 5.8), was added. After mixing for 2 hours, the suspension was filtered. After washing the CM-Sephadex resin 3 times with 0.3% sodium chloride, adsorbed IgG was eluted with 1.4 N sodium chloride. The eluate was clarified, diafiltered and concentrated. D-sorbitol was added and final adjustments were made to yield a solution with composition of about 100 mg/mL IgG, 50 mg/mL D-sorbitol at pH 5.4. The solution was then sterile filtered through sterilized bacterial retentive filter and filled into vials.

The intermediate bentonite step in this Example is useful for further reducing the presence of hypotensive enzymes such as kallikrein and pre-kallikrein activator.

Test Results on Product from Example 1

Test Parameters	
Anti-Complementary Activity (CH_{50}u/mg IgG)	0.34
IgG Purity (%)	100
IgG Content (mg/mL)	112.7
Prekallikrein (% CBER Ref#3)	21
Measles Antibody (% CBER Ref Lot No. 176)	0.67
IgG Molecular Size Distribution by HPLC (%) Monomer (%) Dimer (%) Fragments (%) Aggregates	 82.2 17.4 0.10 0.3
Hepatitis A Antibody (titer)	1:200
Hepatitis B Antibody (titer)	1:1024
IgA (µg/mL)	22
IgM (µg/mL)	16
Plasminogen (ng/mL)	ND
Plasmin (ng/mL)	16
pH	5.4

ND= None Detected

Example 2: Heat Treated and Solvent-Detergent Treated γ-Globulin

One (1) kg of Fr II + III paste was suspended in 4.5 kg of cold water at 0-2°C. After mixing for 1 hour, pH of the suspension was adjusted to 5.0 with 1 N hydrochloric acid. After mixing for 3 hours at pH 5.0, the precipitate was removed by centrifugation. D-sorbitol was added to the centrifugate to a final concentration of 33% and mixed for 1 hour. The pH of the suspension was adjusted to 5.5 and then it was heated for 10 hours at 60°C. After the heat treatment, cold water equal to 3 times the volume of the heat treated solution was added and the diluted solution was cooled to 0-2°C. Fifty percent polyethylene glycol (PEG) 3350 solution was added to a final concentration of 6% PEG. The pH of the 6% PEG suspension was adjusted to 5.7 with 0.5 N sodium hydroxide and the suspension was mixed for 2 hours. Acid wash Celite 535 was added to a final concentration of 1.5% and the suspension was mixed for 1 hour. The precipitate along with the Celite was removed by filtration. The pH of the filtrate was adjusted to 8.8 with 0.5 N sodium hydroxide, and the PEG concentration adjusted to 12% with the addition of 50% PEG solution. The pH of the 12% PEG suspension was readjusted to 8.8, the suspension being mixed for

1 hour and filtered to collect the purified immune globulin paste. About 251 g of purified immune globulin paste was recovered.

Two hundred fifty one grams of purified immune globulin paste obtained as above was suspended in about 1.4 kg of 0.3% sodium chloride solution. After mixing for 1 hour, pH of the suspension was adjusted to 6.0 with 5% acetic acid. After the paste was dissolved completely, 104 grams of DEAE-Sephadex A-50 resin, previously equilibrated with 0.3% sodium chloride at pH 6.0, was added to the solution and mixed for 2 hours. The resin was removed by filtration. The filtrate was further clarified through 0.2 μm filter. Concentration of sodium chloride in the clarified solution was increased to 0.4% by the addition of sodium chloride. Tri-n-butyl phosphate (TNBP) and Polysorbate 80 mixture was added to the solution to give a final concentration of 0.3% TNBP and 1.0% Polysorbate 80. The solution was then incubated for 1 hour at 27°C and left overnight in a cold box at 4°C. Next day, pH of the solution was adjusted to 5.8 and 1.8 kg of CM-Sephadex C-50 resin previously equilibrated with 0.4% sodium chloride at pH 5.8, was added. After mixing for 2 hours, the resin was separated by filtration. After washing the

CM-Sephadex resin 3 times with 0.3% sodium chloride, adsorbed IgG was eluted with 1.4 N sodium chloride. The eluate was clarified, diafiltered and concentrated. D-sorbitol was added and final adjustments were made to yield a solution with composition of about 100 mg/mL of IgG and about 50 mg/mL D-sorbitol. The solution was split into 2 parts, part A and part B. The pH of part A was adjusted to 5.4 and part B was adjusted to pH 4.3. The solutions of part A and part B were then individually sterile filtered through sterilized bacterial retentive filters and filled into vials.

Test Results on Product from Example 2

Test Parameters		
Anticomplementary Activity ($CH_{50}u$/mg IgG)	<0.05	
IgG Purity (%)	100	
IgG Content (mg/mL)	104.2	
Prekallikrein (% of CBER Ref #3)	ND	
Diphtheria Antibody (Antitoxin U/mL)	8.2	
IgG Molecular Size Distribution by HPLC	pH5.4	pH 4.3
(%) Monomer	88.8	97.5
(%) Dimer	10.9	2.3
(%) Fragments	0.3	ND
(%) Aggregates	<0.3	<0.3
Hepatitis A Antibody (titer)	1:100	
IgA (µg/mL)	78	
IgM (µg/mL)	28	
Kallikrein (A_{405})	0.09	
Plasminogen (ng/mL)	<8.4	
Plasmin (ng/mL)	<8.4	

ND= None Detected

Variations of the invention will be apparent to the skilled artisan.

The reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in Australia.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A process for preparing an intravenously administrable gamma globulin solution which comprises:

(a) heat treating an impure gamma globulin solution under time and temperature conditions sufficient for inactivating heat sensitive viruses;

(b) subjecting the heat treated gamma globulin solution to polyethylene glycol fractionation for obtaining a purified gamma globulin solution;

(c) treating the purified gamma globulin solution with an organic solvent for inactivating envelope viruses; and

(d) treating the organic solvent treated gamma globulin with a cationic exchange resin.

2. The process of claim 1 wherein the impure gamma globulin solution is Cohn Fraction I + II + III, Cohn Fraction II + III, Cohn Fraction II + IIIw, or Cohn Fraction II.

3. The process of claim 1 wherein the impure gamma globulin solution is subjected to at least one step of purification prior to the heat treating step (a).

4. The process of claim 1 wherein the heat treating step (a) is carried out at about 50 to 70°C for about 10 to 100 hours.

5. The process of claim 4 wherein the heat treating step (a) is carried out for about 10 hours at about 60°C.

6. The process of claim 1 wherein the polyethylene glycol fractionation is carried out in at least two stages in which impurities are removed as a precipitant in a first stage of polyethylene glycol fractionation and the gamma globulin is removed as a precipitant in a second stage of polyethylene glycol fractionation.

7. The process of claim 1 wherein the organic solvent used in step (c) is an alkyl phosphate.

8. The process of claim 6 wherein the organic solvent used in step (c) is an alkyl phosphate.

9. The process of claim 8 wherein the alkyl phosphate is tri-n-butyl phosphate.

10. The process of claim 1 wherein the organic solvent contains a detergent.

11. The process of claim 9 wherein the organic solvent contains a detergent.

12. The process of claim 1 wherein after step (a) the gamma globulin solution is treated with an anionic exchange resin.

13. The process of claim 12 wherein the anionic exchange resin treatment is prior to step (c).

14. The process of claim 6 wherein a bentonite clarification step is carried out after the first stage of polyethylene glycol fractionation.

15. An intravenously-administrable gamma globulin solution produced by the process of any one of claims 1 to 14.

16. A process according to any one of claims 1 to 14 substantially as hereinbefore described with reference to the examples.

17. An intravenously-administrable gamma globulin solution according to claim 15 substantially as hereinbefore described with reference to the examples.

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